

Fractos Formulation

Due to the lack of information on how much pressure can be tolerated by the BBa_K1904005 complex, the gel is a far better option for the formulation proposal of our project. Gels are usually translucent systems formed by colloidal substances that are capable of forming continuous ordered three dimensional structures.

It is strongly suggested that the formulation of a hydrogel because they can be directly applied on the skin and rinsed afterwards. this has allowed them to be useful in the production of facial cleansers, toothpastes, cosmetics, sunscreen, etc. hydrogels are usually composed by the following¹:

1. **Gelling agent:** usually polymeric substances capable of forming three dimensional structures in liquid solutions. They can be pH dependent or nondependent. Usage of methyl cellulose due to its pH stability from 3 to 11 and its resistance to temperature changes.
2. **Neutralizing agent:** Incorporated to pH dependent gelling agent based formulations. Solutions such as NaOH that act as bases that affect the transparency of the final product.
3. **Humectants:** They can help prevent the evaporation of the water in the formulation and it keeps the formulation from being dry one in contact with the skin. Glycerin is commonly used as it contributes to the expected hydrating actions of these substances on the skin.
4. **Others:** Most formulations contain deionized water, colorants, perfumes, etc. that allow the formulation to offer a more pleasant experience for the user. Other components such as EDTA-Na₂ to avoid the formation of complexes.

However it is important to take into consideration that even if there are several benefits when working with hydrogels, there have also been several difficulties reported with these substances such as their incompatibility with active ingredients that may alter the final pH of the formulation as well as the ionic content of it since this may destabilize the structure of the gel, they tend to get dry easily losing their original texture, they have low penetration on skin and sun exposure may lead to depolymerization and texture loss.

¹ Juvé, J., Viscasillas, A., and del Pozo, A. (2007). *Geles en dermofarmacia: conceptos generales y elementos para su formulación*.

A first approach to the formulation is shown in **Table 1**.

Ingredient	Function
Deionized water	Moisturizer
Glycerin (<10% w/w)	Humectant
Phenonip (mixture of parabens and phenoxyethanol)	Conservative agent
Natrosol 250HX (Hydroxyethylcellulose)	Rheological modifying agent
Fractos protein (BBa_K1904005)	Active agent
Sodium Hydroxide	pH regulator
Fractos blue colorant	Colorant

A second approach, which is common for gel formulations², is given in **Table 2**.

Ingredient	Quantity	Function
Deionized water	100mL	Moisturizer
Triethanolamine	0.4mL	pH regulator
Nipasin	0.1g	Conservative agent
Nipasol	0.05g	Conservative agent
Carbopol 940	0.5g	Rheological modifier agent
Fractos protein (BBa_K1904005)	To be determined.	Active agent
Fractos blue colorant		Colorant

The second approach takes into consideration a better pH regulator, due to the Sodium Hydroxide being a protein aggressive regulator, Triethanolamine acts as a neutral pH regulator, however interactions with the endo-holin complex must be studied. While the conservative agents were proposed as an option for the Phenonip (which is an expensive conservative agent).

Note: We contacted RPH. Pamela Araiza, formulation expert, and she told us that the formulation achieved is enough for the moment since we cannot assign percentages yet because we don't have the protein to experiment with. She approved most of the ingredients in the formulation and we were instructed to research on particular concerns of hers like the availability of parabens and glycerin's physical properties and concentration.

² Cumbreño, S. and Higuero, F. (2004). *Elaboración de emulsiones*. OFFARM. Retrieved from <http://www.elsevier.es/es-revista-offarm-4-articulo-elaboracion-emulsiones-13062391?redirectNew=true>

Costs

To approximate the total costs of the formulation a first approach involving quantities is needed. Quantities can be set for initial purposes, but they are totally dependent on test results done directly on the protein of interest, then the following costs are far from definitive. Costs may only be calculated for the second approach, which includes standard quantities for each component. Each individual cost is calculated from pure reagents. The real cost is set according to the maximum number of gels that can be made, then the total reagent cost is divided by this quantity.

Table 3. Approximate costs for the second approach formulation. All costs are calculated for the smallest presentation of the reagent which tends to have a higher quantity/price relation. Prices set by the provider by August 11, 2016.

Ingredient	Quantity	Total reagent costs (MX)	Presentation / Provider	Costs per individual gel (approximate MX)	Number of formulas
Deionized water	100mL	\$435	1L / Sigma Aldrich	\$43.5	10
Triethanolamine	0.4mL	\$499	100mL / Sigma Aldrich	\$1.996	250
Nipagin (methylparaben)	0.1g	\$361	1000mg (1g) / Sigma Aldrich	\$36.1	10
Nipasol (propylparaben)	0.05g	\$1267	1g / Sigma Aldrich	\$63.35	20
Carbopol 940 (poly acrylic acid)	0.5g	\$949	5g / Sigma Aldrich	\$94.9	10
Fractos protein (Endoholin)	To be determined	Should consider materials and labor.	-----	-----	-----
Fractos blue colorant	To be determined	-----	-----	-----	-----
	Total without the endoholin	\$3511	Total without the endoholin	\$239.846	Cost per unit (MX) / max 10= \$351.10

Fractos formulation protocol (based on the preparation of other gels³)

- 1.- In a clean glass container place 100 mL of deionized water.
- 2.- Gently add the germicide ingredient (Fractos protein extract) in the correct quantity.
- 3.- Mix 0.1 g of Nipasin and 0.05 g of Nipasol and add the mixture to the glass container while mixing.
- 4.-Pulverize thoroughly 0.5 g of Carbopol 940.
- 5.- Add very slowly the pulverized 0.5 g of Carbopol 940, while eagerly mixing, mix until there are no clots left.
- 6.- When there are no Carbopol 940 lumps left, pour the 0.4mL of Triethanolamine, mix gently.
- 7.- Pour the adequate quantity of Fractos blue colorant, mix gently until the color is consistent.
- 8.- Place the whole mixture in the desired container.

*Fractos gel must be kept in fresh, dry places to avoid the corruption of the proteic extract.

³ Profeco. (s.f.). *Elaboración de gel antibacterial*. Retrieved from <http://revistadelconsumidor.gob.mx/wp-content/uploads/2009/06/gel-antibacterial1.pdf>